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Estimation of Biodegradation Potential of Xenobiotic Organic Chemicals

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A method is described to estimate the biodegradation potential of soluble, insoluble, and unknown organic chemicals. The method consists of two stages: (i) generation of a microbial inoculum in a bench scale semicontinuous activated sludge system during which microorganisms are acclimated to test material and the removal of dissolved organic carbon is monitored and (ii) biodegradability testing (CO_2 evolution) in a defined minimal medium containing the test material as the sole carbon and energy source and a dilute bacterial inoculum obtained from the supernatant of homogenized activated sludge generated in the semicontinuous activated sludge system. Removal and biodegradation are measured using nonspecific methods, at initial concentrations of 5 to 10 mg of dissolved organic carbon per liter. Biodegradability data are accurately described by a nonlinear computer model which allows the rate and extent of biodegradation for different compounds to be compared and statistically examined. The evaluation of data generated in the combined removability-biodegradability system allows the biodegradation potential of a variety of xenobiotic organic chemicals to be estimated.

Biological degradation (biodegradation) of organic chemicals can be accurately measured with specific analytical or radiochemical techniques (4, 13). Such techniques allow chemicals to be assayed for biodegradability in a variety of environmental systems at concentrations approaching actual environmental levels. Unfortunately, relatively few chemicals can be assayed with specific techniques because the development of analytical procedures or synthesis of radiolabeled materials is a lengthy and expensive process. Biodegradation information on the vast majority of new and existing chemicals must be generated with nonspecific methods. This fact has been recognized by some of the regulatory agencies responsible for insuring the environmental safety of new chemicals. Recently published biodegradation testing schemes proposed under the Toxic Substances Control Act are based on nonspecific techniques which follow the microbial transformation of organic carbon (3).

Specific methods have been described to measure carbon metabolism in both low- (4-6, 11, 14) and high-population-density systems (1, 6, 15). Many of these methods, however, use a single criterion, often different, to measure biodegradation. This makes it difficult to compare data between methods or to interpret results with respect to possible limitations in a specific test method. For example, in low-population-density systems, ultimate degradation (mineralization to carbon dioxide) and ultimate removal (disappearance of dissolved organic carbon [DOC]) are measured separately with different inocula and different experimental conditions. Removal and degradation numbers cannot be compared, therefore, even though they are complementary measurements and allow biodegradation data to be checked for internal consistency. Acclimation of microorganisms to test material, when conducted, is achieved by exposing a low level of microorganisms to parts per million concentrations of test material over a period of weeks with only trace quantities of added carbon and energy sources present. The limited diversity of microorganisms present initially plus the absence of alternate nutrients do not favor the selection, growth, or adaptation of competent bacteria. Methods for measuring DOC removal in high-population-density systems typically employ very high concentrations of potentially toxic test materials (100- to 1,000-fold above actual environmental concentrations). The use of a separate inoculum prevents comparison of DOC removal data with CO_2 evolution data and makes it more difficult to estimate if removal in the high-population system is primarily due to sorption or bio-oxidation. Statistical programs are not available to define the rate or extent of biodegradation, and it is difficult to determine if extraneous factors like microbial toxicity are

influencing estimates of biodegradation potential.

It was the objective of this study to measure biodegradation of model soluble, insoluble, and unknown compounds in both high- and low-population-density systems using a common inoculum derived from and acclimated in an environmentally relevant system. In addition, emphasis was placed on developing statistical and experimental procedures to allow the rate and extent of biodegradation of low concentrations of test materials to be accurately assayed with nonspecific methods. Experimental conditions were chosen in the laboratory to provide batch simulations of both wastewater treatment and surface water situations. Therefore, estimates of biodegradation potential, defined as the potential for a compound to be mineralized by a heterogeneous population of microorganisms, have relevance to these environmental systems.

MATERIALS AND METHODS

Test materials. Compounds used in this study were representative of soluble, insoluble, and unknown chemical classes (Table 1). Glucose was also used as the positive control. Stock solutions of soluble test materials were prepared at nominal concentrations of 1,000 mg per liter, stored in the dark at 4°C, and assayed for total organic carbon (TOC) with a Beckman 915 Total Carbon Analyzer equipped with a model 865 nondispersive infrared analyzer. Insoluble chemicals were added on a weight or volume basis. All chemicals were reagent grade.

Organisms. A mixed culture of microorganisms was generated in a bench scale semicontinuous activated sludge (SCAS) system. The SCAS system was operated by the Soap and Detergent Association procedure (9) except that effluent samples were analyzed for DOC instead of methylene blue active substances. Mixed liquor suspended solids were adjusted to 2,500 to 3,000 mg per liter. Activated sludge was exposed to increasing levels of test material over a 5-day period (4, 8, 12, 16, and 20 mg per liter). The 20-mg-per-liter concentration was maintained for a total of 3 days, and then removability studies were conducted for the next 5 days in duplicate units at 20 mg per liter (10 to 15 mg of DOC per liter).

Following the removability study, 100 ml of mixed liquor from duplicate SCAS units was composited (200 ml) and homogenized for 2 min at medium speed in a Waring blender at room temperature. The homogenate was allowed to settle for 15 to 30 min, the cloudy supernatant was carefully decanted, and a 1% volume (10 ml per liter) was used as inoculum for biodegradability studies. Since DOC removal could not be measured for the insoluble test materials, isopropyl tetradecanoate (IPTD) and isopropyl palmitate (IPP) SCAS studies were impractical for these compounds. Therefore, inocula for CO_2 evolution testing were derived from different sources. Activated sludge which had acclimated to laboratory conditions (9) but had not been exposed to test material was prepared in the normal manner and used as inoculum for IPTD degradation studies. A 1% volume of settled influent wastewater was used for CO_2 evolution studies of IPP. For both inocula, initial population densities were the same and in the range observed for the standard SCAS inoculum (see below). Bacterial numbers were determined by viable counts (quadruplicate) on standard methods agar containing 8 g of nutrient broth per liter.

Biodegradation assays. Biodegradability (CO_2 evolution) testing was conducted in a closed shake flask system containing the test material as the sole carbon and energy source. Each flask contained 1 liter (final volume) of standard BOD medium (2) with two times the level of phosphate buffer, four times the level of $FeCl_2$, and 40 mg of $(NH_4)_2SO_4$ (BOD-PLUS) per liter of high-quality water (18 Mohm). Experiments with different growth media indicated that BOD-PLUS contained sufficient nutrients to support metabolism of the level of compounds tested. The BOD-PLUS contained low levels of background carbon and lacked alternate carbon and energy sources which simplified DOC and CO_2 measurements. Flasks were aerated with compressed air that had been scrubbed free of CO_2 by passage through a series of six 1-liter plastic bottles each containing 700 ml of 10 N NaOH. The CO_2-free air was routed to a manifold system which fed the headspace of twelve 2-liter Erlenmeyer flasks. Exit gas from the flasks was passed through a series of three 125-ml bottles each containing 100 ml of 0.024 N $Ba(OH)_2 \cdot 8H_2O$ to trap the CO_2 produced during metabolism of the test material. The flow rate of air to the scrubbing bottles was regulated to insure that the scrubbing capacity of the NaOH and $Ba(OH)_2$ scrubbers was not exceeded. Flasks were continuously agitated at 120 (±10) rpm on a rotary platform shaker (New Brunswick Scientific Co., New Brunswick, N.J.) and incubated in the dark at 24 ± 2°C in a constant-temperature room.

Test materials were assayed singly at replicate concentrations (10 and 20 mg per liter nominal) to determine the accuracy of rate constant predictions and to provide information on the kinetics of degradation. A glucose control (20 mg per liter) and a blank containing no carbon source were also included with each set. Flasks were aerated overnight with CO_2-free air to remove residual CO_2 before test material and inoculum were added. Periodically, the $Ba(OH)_2$ absorber nearest the flask was removed to determine the amount of CO_2 trapped as $BaCO_3$. The remaining traps were moved one position closer, and a fresh trap was placed at the far end. With each change of traps a 100-ml $Ba(OH)_2$ blank was included to monitor endogenous CO_2 production from the control flask containing no carbon and energy source. Testing was continued until CO_2 production reached a plateau value suitable for regression analysis, and then 1 ml of concentrated HCl was added to terminate the experiment and convert residual carbonates to CO_2. Acidification did not result in significant amounts of CO_2 being released.

Analytical procedures. The amount of CO_2 trapped as $BaCO_3$ in the $Ba(OH)_2$ absorbers was determined by titrating the contents of each absorber with standard 0.05 N (±0.001) HCl (Fisher Scientific

TABLE 1. *Chemical characterization of test compounds*

Compound			Name and structure
Class	Type	Abbreviation	
Soluble	Anionic surfactant	LAS I LAS II	Linear alkyl benzene sulfonate (para-substituted benzene ring bearing R, with $SO_3^-Na^+$) LAS II $R_{ave} = C_{11.8}$, mean ∅ position = 3.70 LAS I $R_{ave} = C_{11.7}$, mean ∅ position = 3.93
	Nonionic surfactant	AE I AE II	Linear primary alcohol ethoxylate $CH_3—(CH_2)_x—O—(C_2H_4O)_yH$ AE I x = 13, 14; y = 7 average AE II x = 11, 12; y = 6.5 average
	Zwitterionic surfactant	Zwit I	Zwit I $R—N^{\oplus}(R^1)_2—(CH_2)_n—CH(OH)—(CH_2)_n—SO_3^{\ominus}$ $R = C_{10} - C_{18}$ $R^1 = <C_5$ $n = <5$
		Zwit II	Cetyldimethylpolyoxyethylenesulfate $CH_3(CH_2)_{15}—N^{\oplus}(CH_3)_2—(C_2H_4O)_{12}SO_3^{\ominus}$
Insoluble	Fatty ester	IPTD IPP	$CH_3—(CH_2)_n—C(=O)OCH(CH_3)_2$ IPP, $n = 14$ IPTD, $n = 12$
Unknown	Cationic polymer	CAP I	Complete molecular structure unknown – mol wt ~20,000, 37.5% carbon
		CAP II	Complete molecular structure unknown – mol wt ~20,000, 50.2% carbon

Co., Pittsburgh, Pa.) to a phenolphthalein endpoint. The difference in volume of titrant between the blank and test flasks was converted to milligrams of CO_2 by the following equation: milligrams of CO_2 = (0.025 mmol per ml) × milliliters of HCl × (44 mg of CO_2/millimole).

The actual amount of CO_2 evolved after correction for blank controls was expressed relative to the total amount of CO_2 which could be produced upon complete conversion of all test carbon to CO_2 by the following: percent total CO_2 = [milligrams of CO_2 produced/(milligrams of material added × theoretical CO_2)] × 100, where theoretical CO_2 = [(number of carbon atoms in material × molecular weight of CO_2 = 44)]/molecular weight of material.

In the case of chemicals for which the molecular structure was unknown, results were based on the carbon content of the molecule and were expressed as

percent TOC-CO_2 (amount of TOC converted to CO_2) by the following equation: percent TOC − CO_2 = {milligrams of CO_2 produced/[milligrams of TOC added × (44/12)]} × 100.

DOC analyses were performed on samples of SCAS effluent (23.5-h retention time) and BOD-PLUS medium after acidification to pH 2 and centrifugation at 27,000 × *g* for 20 min. About half the supernatant was carefully drawn off and analyzed directly for DOC or purged in an N_2 stream before analysis. Nitrogen purging was necessary during DOC analysis of SCAS effluents since the tap water used for making the synthetic feed contained considerable amounts of inorganic carbonates. However, since background carbon levels in the 18-Mohm high-quality water were <1 ppm, purging was not necessary on a routine basis for the CO_2 evolution test. Test materials were not volatilized by the purging procedure. The DOC values for the test units were corrected for background carbon by subtracting the values in control units containing no test material. The percentage of DOC removed after correction for controls was calculated by the following formula with the assumption that DOC and TOC were equivalent for soluble materials: percent DOC removed = [(milligrams of TOC added per liter − milligrams of DOC remaining per liter)/milligrams of TOC added per liter] × 100.

Statistical analysis of data. The DOC removal data for duplicate SCAS units were subjected to an analysis of variance (ANOVA), by using the duplicate units to estimate inherent variability over the test period. When a significant *F*-ratio (95% confidence) was observed in the ANOVA, the least significant difference was computed to determine if significant differences existed between test compounds (10). The CO_2 evolution data from multiple experiments were analyzed by various empirical models and fit to a nonlinear regression analysis of the following form:

$$y = \begin{cases} 0 \text{ for } x \leq c \\ a(1 - e^{-b(x-c)}) \text{ for } x > c \end{cases}$$

where y = percent total CO_2, x is the time in days, and a = asymptote of CO_2 evolution curve (percent total CO_2), b = rate constant for CO_2 evolution (day^{-1}), and c = lag time before CO_2 evolution begins (day). The 95% confidence intervals were determined for constants, and plots were made by computer with 95% confidence intervals indicated.

RESULTS

Preparation of inocula. A 20-fold increase in the number of colony-forming units (CFU) was observed after activated sludge (2,500 to 3,000 mg of mixed liquor suspended solids per liter) was homogenized for 2 min and allowed to settle for 30 min (Fig. 1). The number of CFU/ml in the settled supernatant remained constant up to 4 min of homogenization, which indicates that extended homogenization does not adversely affect cell viability nor does it increase total cell numbers beyond the 2-min level. Similar results were obtained when activated sludge obtained from a residential treatment facility was homogenized (data not shown). Homogenization of six different batches of sludge generated over an 8-month period resulted in a fairly constant number of CFU/ml in the settled supernatant, ranging from 10^6 to 2×10^7 CFU/ml. A 1% (vol/vol) inoculum, therefore, was sufficient to achieve reproducible population densities typical of surface waters (10^4 to 10^5 CFU/ml) in CO_2 shake flasks, while keeping background carbon levels and endogenous metabolism low to maintain analytical sensitivity.

Soluble compounds. The rate and extent of CO_2 evolution for linear alkyl benzene sulfonate I (LAS I) were high (Fig. 2) when the average alkyl chain length was $C_{11.7}$ and phenyl distribution averaged 3.93. The extent of DOC removal in the SCAS acclimation system was also high, averaging 91.6% over a 5-day period (Table 2). The extent of CO_2 evolution was reduced 40 to 50%, however, when a higher percentage of C_{13} and C_{14} alkyl chain lengths was present in

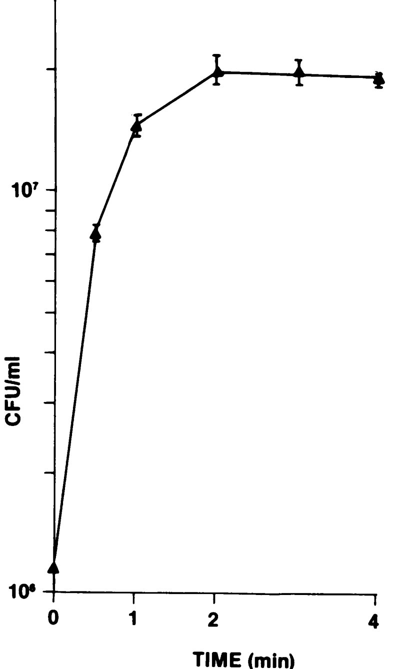

FIG. 1. *Effect of mechanical homogenization on the number of CFU in the supernatant of settled activated sludge. Approximately 200 ml of mixed liquor suspended solids (~3,000 mg per liter) from the SCAS system were homogenized for the times indicated before viable counts were made on the settled supernatant. The 0 time value represents the number of CFU/ml in the settled supernatant before homogenization.*

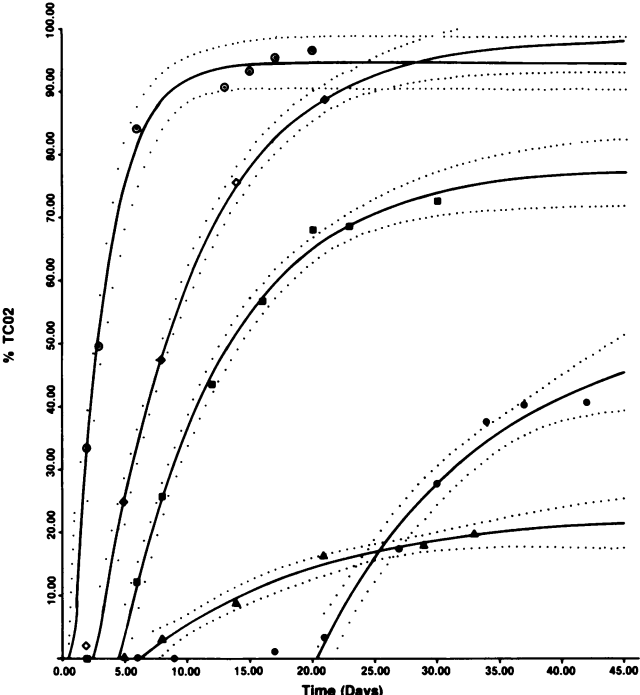

FIG. 2. *Kinetics of CO_2 evolution during metabolism of various water-soluble surfactants. The actual data have been fit by computer to a nonlinear empirical model* $y = a(1 - e^{-b(t-c)})$ *(see Table 2) by iterative techniques.* ◇, *AE I (9.3 mg per liter);* ■, *LAS I (8.3 mg per liter);* ●, *Zwit I (9.3 mg per liter);* ▲, *Zwit II (9.0 mg per liter); a representative glucose control is included for comparative purposes* (○, *20 mg per liter). Dotted contours are 95% confidence limits of true means.*

the LAS sample (LAS II) and the average phenyl position moved toward a more terminal position (Table 3). Lag times preceding LAS II degradation were also 3 to 10 days longer than those preceding LAS I. However, the extent of DOC removal in the SCAS system was high and statistically the same for both LAS I and LAS II.

The rate and extent of CO_2 evolution were high for two alkyl ethoxylates (AE I and AE II), approaching theoretical limits (Table 2). DOC removal in the SCAS system was also essentially complete. Rate constants for CO_2 evolution from the nonionic surfactants were reproducible in separate experiments, and DOC could not be detected in the growth medium at the end of either experiment (resolution limit, 0.5 mg of DOC per liter).

The extent of CO_2 evolution in shake flasks and DOC removal in the SCAS system for two zwitterionic surfactants indicate only partial degradation of these compounds (Fig. 2). Estimated CO_2 figures for zwitterionic I and II (Zwit I and II) were about 55 and 25%, respectively, which are in reasonable agreement with the amount of DOC removal observed in the SCAS system (56.5 and 32.8%). For both compounds, the remaining organic carbon which was not converted to CO_2 could be found in the growth medium as DOC at the end of the experiment (Table 2). The actual degradation of Zwit I continued beyond the 42-day test period since predicted and observed CO_2 numbers are slightly different.

Unknown compounds. The rate and extent of CO_2 evolution for two soluble cationic polymers could not be determined by using theoretical considerations since the molecular structure of neither material was known. Biodegradability calculations were therefore based on the amount of organic carbon in the compounds converted to CO_2, as described above. The CAP (cationic polymer) I and CAP II samples tested evolved about 30 and 0% of their TOC as CO_2, respectively (Fig. 3, Table 2). The remaining organic carbon was detected as DOC in the growth medium at the end of the experiment. The low CO_2 results are in contrast to the results in the SCAS

TABLE 2. *Biodegradation of soluble surfactants and unknowns*

Compound	Length of expt (days)	Biodegradability: Observed: Total CO_2 (%)	Observed: DOC removed[a] (%)	Predicted: a (Percent total CO_2)	Predicted: b (day^{-1})	Predicted: c (days)	Predicted: R^2	SCAS removability (% DOC removal)[b]
LAS I-10	30	72.9	81	78.2 ± 2.4[c]	0.11 ± 0.01	4.6 ± 0.3	0.998	
LAS I-20		67.3		77.5 ± 4.9	0.09 ± 0.01	5.5 ± 0.3	0.995	91.6 ± 2.4
LAS II-10	30	48.4	55	50.0 ± 1.0	0.21 ± 0.02	7.3 ± 0.2	0.998	
LAS II-20		37.0		40.9 ± 1.8	0.17 ± 0.02	15.2 ± 0.2	0.998	92.6 ± 3.1
Glucose-20		86.0	100	86.1 ± 2.1	0.20 ± 0.02	0 ± 0.4	0.985	
AE I-10	29	95.0	100	98.7 ± 1.7	0.13 ± 0.01	2.7 ± 0.2	0.999	
AE I-20		99.8		99.9 ± 8.6	0.10 ± 0.02	1.5 ± 0.6	0.986	97.9 ± 5.4
AE II-10	24	98.7	100	93.1 ± 14.9	0.13 ± 0.07	2.0 ± 1.8	0.932	99.7 ± 5.3
AE II-20		86.5		96.4 ± 8.0	0.09 ± 0.02	1.8 ± 0.4	0.991	
Glucose-20		96.5	100	94.6 ± 1.5	0.38 ± 0.05	0.9 ± 0.2	0.992	
Zwit I-10	42	41.0	49	55.3 ± 9.1	0.07 ± 0.02	20.0 ± 0.7	0.987	56.5 ± 5.1
Zwit I-20		—[d]	—	—	—	—	—	
Zwit II-10	33	19.7	25	23.2 ± 2.6	0.07 ± 0.02	6.3 ± 0.8	0.993	32.8 ± 8.9
Zwit II-20		17.4		25.9 ± 4.0	0.05 ± 0.01	11.4 ± 0.6	0.998	
Glucose-20		97.7	100	95.6 ± 1.6	0.16 ± 0.02	0.06 ± 0.3	0.992	
CAP I-10	35	32.3[e]	31	31.3 ± 0.7	0.34 ± 0.03	4.5 ± 0.10	0.997	101.3 ± 5.1
CAP I-20		26.8		26.6 ± 0.2	0.28 ± 0.01	4.3 ± 0.05	1.000	
CAP II-10	35	0.0	0	—	—	—	—	
CAP II-20		0.0		—	—	—	—	96.1 ± 6.8
Glucose-20		92.0	100	87.7 ± 2.3	0.23 ± 0.02	0	0.965	

[a] Average removal.
[b] Five-day average, ± standard deviation; ANOVA showed a significant compound effect, least significant difference = 3.8.
[c] ± Values are standard errors of parameter estimates.
[d] Insufficient data for determination of constants.
[e] Percent TOC converted to CO_2.

TABLE 3. *Chain length and phenyl distribution of LAS samples*

Compound	Phenyl position (%)[a]: 2	3	4	5	6, 7	Chain length (%)[a]: C_{10}	C_{11}	C_{12}	C_{13}	C_{14}
LAS I	23.6	19.8	18.0	16.9	21.4	7.3	26.5	56.7	9.0	0.4
LAS II	31.5	18.6	16.5	18.4	15.3	9.5	29.2	37.7	19.0	4.9

[a] The percent figures normalized to 100% based on identified gas chromatographic peaks measured as described (12) with modifications (Q. W. Osburn and R. L. Downey, PS & DD, Procter and Gamble Co., Cincinnati, Ohio, personal communication).

system where essentially 100% DOC removal was observed. The combined results indicate that removal in the SCAS system is primarily due to physical-chemical sorption processes, a result which is consistent with the high affinity of these materials for organic solids. Since CAP I was partially metabolized at a rapid rate (higher than the glucose control), part of the sorbed material can also be assumed to undergo mineralization.

Water-insoluble compounds. The rate and extent of CO_2 evolution were high for two water-insoluble fatty esters, IPP and IPTD, when they were present as sole carbon and energy sources

TABLE 4. *Biodegradation of insoluble compounds*

Compound	Observed		Predicted				
	Total CO_2 (%)	% DOC removed	a[a] (% Total CO_2)	b[a] (day^{-1})	c[a] (days)	R^2	Rate (mg of CO_2/liter per day)[b]
IPP-10	89.9[c]	—[d]	84.2 ± 2.4	0.27 ± 0.03	0.8 ± 0.20	0.980	6.4 ± 0.7
IPP-20	79.9		75.0 ± 1.9	0.30 ± 0.04	0.3 ± 0.20	0.975	12.6 ± 1.3
IPTD-10	81.7	—	82.9 ± 0.4	0.33 ± 0.01	1.0 ± 0.10	0.998	7.6 ± 0.3
IPTD-20	90.0	100	89.6 ± 1.1	0.24 ± 0.02	0.8 ± 0.20	0.994	11.9 ± 1.0
Glucose-20	93.0		90.8 ± 1.6	0.24 ± 0.02	0.1 ± 0.40	0.984	

[a] See text for definitions of a, b, and c.
[b] Maximum rate at point t = c.
[c] Results after 25 days.
[d] —, Could not be determined.

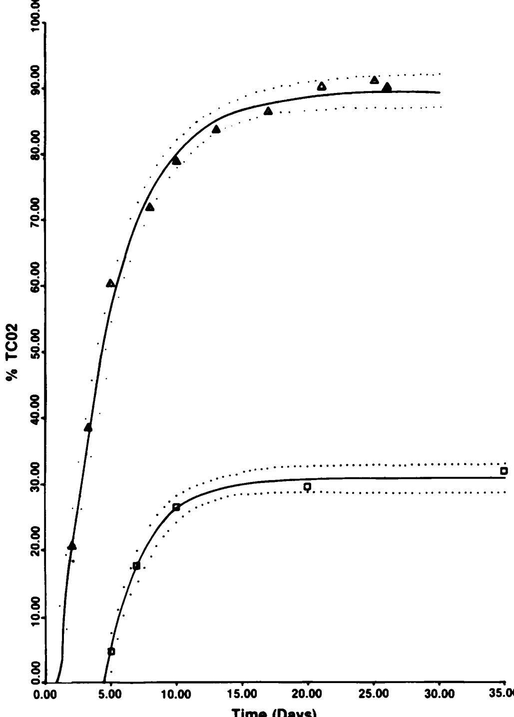

FIG. 3. *Kinetics of CO_2 evolution during metabolism of a water-insoluble compound (IPTD) and a soluble unknown (cationic polymer). △, IPTD, 9.9 mg/liter; □, CAP I, 3.7 mg of TOC per liter. Dotted contours are 95% confidence limits of true means.*

(Fig. 3). Removal of DOC in the CO_2 and SCAS systems was not measured for either compound since they were physically removed by centrifugation or filtration required for DOC analysis. The rate of CO_2 evolution (milligrams of CO_2 per liter per day) for each compound was higher at 20 mg per liter than at 10 mg per liter (Table 4), even though the apparent solubilities of both IPP and IPTD were <10 mg per liter. Mineralization of these compounds, therefore, was not totally dependent on the concentration of material in solution. An increase in the rate of CO_2 evolution at substrate concentrations above the solubility limit has also been observed for a number of other insoluble chemicals (data not shown), including those that form particulate suspensions as well as water emulsions.

DISCUSSION

In this study, CO_2 production and DOC removal were measured in a low-population-density system, and DOC removal was measured in a high-population-density system to estimate the biodegradation potential of model soluble, insoluble, and unknown compounds. Population levels in the CO_2 shake flask system are typical of surface waters (~10^5 CFU/ml), and biodegradation rate data are relevant to these types of dilute environmental systems. Biomass levels in the SCAS system, however, are significantly higher than in the CO_2 system and are typical of an activated sludge treatment facility (~3,000 mg of mixed liquor suspended solids per liter). The 24-h aeration period is somewhat longer than the hydraulic retention time of most activated sludge treatment facilities and most closely approximates an extended aeration system. The 24-h hydraulic retention time is necessary, however, to reduce background DOC levels of the nutrient feed to sufficiently low levels so that DOC removal from the test material can be detected from background.

The combined CO_2-SCAS system can be applied to a variety of compounds since nonspecific methods are used to follow carbon metabolism. Carbon dioxide evolution is measured in a dilute synthetic medium containing the test material as the sole carbon and energy source. The low population density in the CO_2 system helps to reduce endogenous CO_2 production and nonspecific sorption effects so that milligram quantities

of CO_2 produced during metabolism can be accurately measured. The amount of DOC removal observed in the CO_2 shake flask is also determined for mass balance purposes. DOC removal is not used as a primary measure of biodegradation since it does not allow actual bio-oxidation to be determined and is less sensitive than CO_2 evolution measurements.

Two major disadvantages of the CO_2 system are that biodegradation is measured in the absence of alternate carbon and energy sources, and the low level of microorganisms can be susceptible to toxic properties of the test material. Therefore, DOC removal studies in the SCAS system are conducted with multiple carbon sources and a high biomass level typical of activated sludge treatment facilities. Because multiple carbon sources are present in the SCAS system and endogenous metabolism is high, the amount of CO_2 produced from the test material cannot be detected from background. The amount of DOC removal due to bio-oxidation, therefore, cannot be differentiated from that due to sorption. However, by comparing DOC removal with CO_2 evolution in a two-stage procedure using the same basic population of microorganisms, the capacity of that heterogenous population to metabolize a compound can be determined, and the biodegradation potential can be estimated.

The combined SCAS-CO_2 system using high and low population levels tends to compensate for limitations of either system used singly. Disparate results from the two systems should be critically examined to determine how they affect estimates of biodegradation potential. For example, high DOC removal in the SCAS system and low CO_2 evolution may indicate that a chemical is toxic in a low biomass system. For LAS II, long lag times and reduced CO_2 evolution suggest that this particular sample has reached a critical toxicant-biomass ratio in the CO_2 system. Toxic effects were not observed for an LAS sample which differed only in the distribution of isomers or in the SCAS system with a higher biomass level. Low CO_2 production and high DOC removal, as observed for the cationic polymers, may also reflect the high sorptive properties of a chemical, which result in overestimates of biodegradability. Conversely, reasonable agreement (within 10 to 15%) between CO_2 evolution and DOC removal provides clear evidence of the biodegradability of a material in both surface water and treatment situations. For AE I and II, Zwit I, and Zwit II, agreement between CO_2 and SCAS results allows these compounds to be placed in categories of high, medium, and low degradability, respectively.

Since DOC removal includes the amount of test material incorporated into biomass as well as that oxidized to CO_2, the amount of DOC removed is generally greater on a percent basis than the amount of CO_2 evolved. However, the difference is not as great as might be predicted, based on classical growth kinetics (8). The CO_2 evolution data for AE I, II, and glucose indicate that substantial amounts of CO_2, approaching theoretical limits, can be produced from compounds when they are present as sole carbon and energy sources. The CO_2 evolution curves have no obvious discontinuities before high plateau values are reached, and the CO_2 data at all sampling points can be accurately described by a single rate constant, which does not increase as the concentration of test material increases. These results indicate that apparent microbial growth yields are low in the CO_2 system at the substrate concentrations used. Low net growth can be attributed to high maintenance energy requirements (7, 8) which result in catabolism of large amounts of substrate carbon to CO_2 and a smaller level of assimilation into new cells. High CO_2 conversion efficiencies under low net growth conditions make bio-oxidation an accurate measure of the rate and extent of actual biodegradation and allow CO_2 evolution to be compared with DOC removal.

Removal and biodegradation can be measured in the SCAS and CO_2 system, respectively, for chemicals of unknown structure if the amount of organic carbon in the chemical can be measured. Although TOC analyses are generally limited to soluble chemicals, water-insoluble compounds can also be analyzed by combustion techniques if homogeneous aqueous emulsions or suspensions can be made. Water-insoluble chemicals can also be assayed for biodegradability in the CO_2 system as long as degradation rates are not limited by the concentration of material in solution. Even unknown insoluble compounds can be tested if the amount of TOC in the chemicals can be measured. However, DOC removal cannot be measured in the SCAS or CO_2 system for water-insoluble compounds since unrealistically high removal values are caused by sample preparation techniques.

The rate and extent of CO_2 production for organic chemicals tested in this study are accurately described by an empirical nonlinear regression model. The constants generated by the empirical model characterize the CO_2 evolution curve for a particular material, and good correlations are observed between predicted and experimental curves. Basically, the constants allow the separation of the biodegradation process into three related but distinct categories: a met-

abolic lag period where the appropriate metabolic mechanisms are being selected or an active population is being produced or both, a period of maximal degradation where the molecule is being actively metabolized, and a plateau period defining the extent of metabolism of the molecule.

The extent of degradation as defined by the asymptote of the CO_2 evolution curve, a, gives a time-independent indication of how much of the molecule is potentially metabolizable. Since the asymptote essentially defines CO_2 evolution at infinite time, the problem of when to end testing is effectively dealt with. When evaluated jointly with the amount of DOC removal observed in the SCAS system, the asymptotic value also allows the biodegradation potential for a chemical to be estimated. The asymptote or extent of degradation is determined using all the CO_2 evolution data points, which helps to statistically define and minimize the effects of experimental error.

The rate constant, b, defines the rate of change of degradation over time and therefore provides a concentration-independent estimate of how fast degradation is occurring. Rate constants facilitate the comparison and analysis of data between experiments. They can be normalized for population size or for rate constants of standard compounds or both to allow more valid comparisons to be drawn between different materials. Rate constants also facilitate kinetic studies to determine the effect of concentration on the rate of degradation and allow calculation of degradation half lives.

The lag times observed before degradation begins, c, provide information on the character of the microbial population as well as the ability of that population to metabolize a chemical as a sole carbon and energy source. Long lag times can indicate that a chemical is toxic at a particular chemical-to-biomass ratio or that the percentage of the total initial population metabolizing the chemical is very small. Both factors can be serious limitations in any biodegradability test system where low population densities and high concentrations of test material are present, and they can be important considerations when attempting to interpret biodegradability results.

Ideally, estimates of biodegradation potential should be based on estimates of microbial metabolism which are unencumbered by testing artifacts. The SCAS-CO_2 system described in this study attempts to provide such estimates by using nonspecific methods which are broadly applicable to soluble, insoluble, and unknown organic chemicals. Disparate results between the two systems are an indication that factors unrelated to carbon metabolism are influencing estimates of biodegradation potential.

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